Risk of Benign Gynecologic Tumors in Relation to Prenatal Diethylstilbestrol Exposure

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OBJECTIVE: To investigate the association between prenatal diethylstilbestrol (DES) exposure and risk of benign gynecologic tumors.

METHODS: We conducted a collaborative follow-up study of women with and without documented intrauterine exposure to DES. We compared the incidence of self-reported ovarian cysts, paraovarian cysts, and uterine leiomyomata confirmed by medical record in DES-exposed and unexposed women.

RESULTS: A total of 85 cases of uterine leiomyomata and 168 cases of ovarian or paraovarian cysts were confirmed histologically. After adjustment for age, no association was found between prenatal DES exposure and ovarian cysts or uterine leiomyomata. Prenatal DES exposure was positively associated with paraovarian cysts.

CONCLUSION: The present results do not support the hypothesis that prenatal DES exposure increases risk of uterine leiomyomata or ovarian cysts. Prenatal DES exposure was associated with an increased risk of paraovarian cysts, but detection bias cannot be ruled out as an explanation of this finding. (Obstet Gynecol 2005;105:167–73. © 2005 by The American College of Obstetricians and Gynecologists.)

LEVEL OF EVIDENCE: II-2

Diethylstilbestrol (DES) is a synthetic estrogen that was prescribed to more than 2 million pregnant women during the 1940s to 1960s to prevent adverse pregnancy outcomes. It was later discovered to be associated with the occurrence of vaginal and cervical adenocarcinoma in the female offspring.[1] Prenatal exposure to DES has been associated with poor reproductive outcomes[2–4] and abnormalities of the uterus and reproductive tract in humans.[2,5–7] In studies of the CD-1 outbred mouse, a range of reproductive effects has been linked to perinatal DES exposure, including smooth muscle changes in the uterus,[8] a low but significantly increased incidence of leiomyomata (5%),[8–10] and benign and malignant epithelial tumors throughout the reproductive tract.[8]

The potential effects of prenatal DES exposure on the incidence of benign tumors of the reproductive tract have not been adequately explored in humans. Ovarian cysts and uterine leiomyomata are a major source of gynecologic morbidity in reproductive-aged women. Both conditions are included among the top 5 leading causes of hospitalizations for gynecologic conditions unrelated to pregnancy in women aged 15–44 years.[11] Moreover, uterine leiomyomata are the leading indication for hysterectomy among women of all ages in the United States.[12] Although little is known about the cause of these benign tumors, steroid hormones are thought to play an important role.[13,14] Diethylstilbestrol, which possesses estrogenic and endocrine-disrupting activity, may contribute to an increased incidence of these tumors by way of hormone-dependent pathways. To explore the hypothesis that prenatal DES exposure is associated with an increased risk of benign reproductive tract tumors later in life, we assessed the incidence of these tumors in a large cohort of DES-exposed and unexposed women.

MATERIALS AND METHODS

A collaborative follow-up study of DES-exposed and unexposed women of the same ages has been ongoing since 1992.[15] For the purposes of the present analysis, 2 cohorts are included in this combined follow-up study. The methods of the original studies from which these cohorts were assembled have been described else-

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where.[16,17] Briefly, the largest cohort consists of women enrolled in the National Cooperative Diethylstilbestrol Adenosis (DESAD) study during the mid-1970s.[16] DES-exposed subjects were identified by prenatal record review at 5 centers. Additional DESAD cohort members who were referred by physicians or self-referred are not included in the present analysis. Women not exposed to DES in utero were randomly selected at the same time and from the same record sources as the exposed women (based on a review of prenatal records and hospital delivery information) or were sisters of the exposed women; the prenatal records of their mothers did not note any exposure to sex hormones (steroidal or nonsteroidal, estrogen, progestins, androgens, or gonadotropins) during the prenatal period. National Cooperative Diethylstilbestrol Adenosis study participants were followed up with clinical examinations through 1980 and by periodic mailed questionnaires since then.

The second cohort (Dieckmann) includes women whose mothers participated in a randomized clinical trial of the efficacy of DES during pregnancy in the early 1950s.[18] In 1974, attempts were made to trace all subjects in this cohort, and 83% of exposed and 77% of nonexposed subjects responded to a questionnaire.[19] Participants from the Dieckmann cohort have been followed up by periodic mailed questionnaire.[20]

The Dieckmann cohort was exposed to high cumulative doses of DES (median cumulative dose approximately 12 g), following the regimen recommended by Smith and Smith.[21] Exposure in the DESAD cohort was difficult to estimate due to incomplete information from medical records, but ranged from a median cumulative dose of approximately 1.5 g at Baylor College of Medicine (Houston, TX) and the Mayo Clinic (Rochester, MN) to 4.5 g at the Boston Lying In Hospital (Boston, MA).[16]

In 1994, the cohorts were combined and all participants were mailed a detailed questionnaire eliciting data on gynecologic surgeries as well as reproductive and contraceptive history, lifestyle factors, medication use, and health care use. In 1997, a shorter follow-up questionnaire was mailed to ascertain new occurrence of disease. Some women were not approached during the 1994 follow-up, either because they were not successfully traced or because they were unwilling to participate in previous follow-ups. Among women mailed a 1994 questionnaire, response was 88% for both exposed and nonexposed women,[15] and the present analyses are restricted to these women. The 1994 questionnaire was completed by 1,811 exposed women (n = 1,522 from DESAD; n = 289 from Dieckmann) and 876 unexposed women (n = 624 from DESAD; n = 252 from Dieckmann). These numbers represent 80% of the exposed subjects and 80% of unexposed subjects originally identified from review of medical records of the DESAD project and 70% of exposed and 64% of unexposed subjects from the Dieckmann cohort. The study protocol was approved by the human subjects review boards at the 5 field centers and the National Cancer Institute. Women gave informed consent by completing and returning the mailed questionnaires.

Table 1. Confirmation of Benign Gynecologic Conditions by Medical Record

	Exposed	Unexposed
Ovarian or paraovarian cysts		
Self-report of cyst	156	62
Records obtained	99 (64)	41 (66)
Diagnosis confirmed	75 (76)	32 (78)
Confirmed cysts from other reports	49	12
Total confirmed cysts	124	44
Uterine leiomyomata		
Self-report of leiomyoma	85	44
Records obtained	62 (73)	33 (75)
Diagnosis confirmed	40 (65)	24 (73)
Confirmed leiomyoma from other reports	15	6
Total confirmed leiomyomata	55	30

Values are number and (percentage).

We sought medical records from all respondents who answered positively to the question "have you ever had an operation for a benign (noncancerous) tumor or cyst of the uterus, fallopian tubes, or other part of the reproductive tract?" on the 1994 or 1997 questionnaire. In addition, questionnaire data were elicited on the affected organ, the exact diagnosis, the year of surgery, and the name and address of the physician or hospital at which the surgery took place.

We attempted to validate self-reports of benign gynecologic tumors, including cysts, as described below. Medical records were obtained for similar proportions of exposed and unexposed participants (Table 1): cysts, 64% exposed compared with 66% unexposed; uterine leiomyomata, 73% exposed compared with 75% unexposed. Three gynecologists, blinded to the exposure status of the subjects, reviewed surgery and pathology reports from centers other than their own (K.L.N., R.H.K., and A.L.H.). In the present study, we included all cysts that could be classified into the following histologic types: functional cyst (including follicular and corpus luteum cysts); cystadenoma (serous or mucinous) or simple cyst; endometrioma (chocolate cyst); benign cystic teratoma (dermoid cyst); or paraovarian cysts, defined to include hydatid, paratubal, or any other type of cyst believed to originate from remnants of the most cephalic portion of the müllerian or wolffian ducts. Be-

cause it is virtually impossible for pathologists to determine whether cysts lined by cuboidal cells with rare cilia are müllerian or wolffian in origin, we combined these histologic types in the present analysis. Our outcome definition excluded women with polycystic ovarian disease.

Among those for whom we could obtain medical records, 76% of the reported ovarian or paraovarian cysts and 67% of the reported uterine leiomyomata were confirmed by medical record, and this percent did not vary appreciably by exposure status (Table 1). An additional 61 ovarian cysts and 21 uterine leiomyomata were confirmed from reports of other gynecologic surgeries and were included in the present analysis. The present analysis includes only diagnoses that could be confirmed by medical record. Women who reported the occurrence of a gynecologic tumor but whose records were not reviewed were excluded from analysis (n = 80 exposed, n = 28 unexposed). The main reason for the inability to review medical records was the extended length of time between report of condition and the initial request for records; many hospital records were no longer available.

Separate analyses were carried out for cysts and uterine leiomyomata. We calculated person-years of follow-up for each subject from date of birth until the earliest of the following events: date of surgery for uterine leiomyomata or cyst, date of hysterectomy (for analyses of uterine leiomyomata), date of bilateral oophorectomy (for analyses of cysts), date of death, or date of last known follow-up. In analyses of cysts, if women reported multiple cysts at different times, person-years were accrued until the earliest cyst diagnosis. Women were permitted to contribute an event to more than one case group. Of the 37 cases with more than one type of cyst, 36 reported the same date of surgery for their cysts. A total of 1,731 exposed women (contributing 74,811 person-years) and 848 unexposed women (contributing 37,418 person-years) were included in the analyses of uterine leiomyomata. The same number of exposed and unexposed women contributed 74,926 person-years and 37,752 person-years, respectively, to the analyses of cysts.

We calculated the median age of surgery for each benign tumor type and tested differences between exposure groups using the nonparametric Wilcoxon rank sum test.[22] Incidence rates were calculated by dividing the number of incident cases in a given exposure group by the person-time contributed by that group. Incidence rate ratios were computed as the ratio of the incidence rate in the DES-exposed to the incidence rate in the unexposed. Analyses were carried out using SAS statistical software (SAS Institute, Cary NC).[23] We used Cox regression models[24] stratified by age (in 1-year intervals) to estimate incidence rate ratios and 95% confidence intervals (CIs) for reproductive tumors comparing exposed to unexposed women. Age was treated as a time-dependent variable. We used the Anderson-Gill data structure to update age over time[25] and the "exact" option to handle tied event times.[26]

Because our parameter estimates did not materially change after adjustment for age at menarche, parity, smoking, use of oral contraceptives, and education (as measured on the 1994 questionnaire), or after taking into account possible correlation within study centers or cohorts, we present only age-adjusted models. Departures from the proportional hazards assumption (ie, a constant incidence rate ratio over time) were tested by the likelihood ratio test, comparing models with and without

Table 2. Characteristics of Women With and Without Prenatal Exposure to Diethylstilbestrol (1994)

Characteristic	Exposed (n = 1,731)	Unexposed (n = 848)
Year of birth		
Before 1950	413 (23.8)	172 (20.3)
1950–1954	786 (45.4)	473 (55.8)
1955–1959	316 (18.3)	180 (21.2)
1960 or later	216 (12.5)	23 (2.7)
Study cohort		
National Cooperative Diethylstilbestrol Adenosis study	1,456 (84.1)	609 (71.8)
Dieckmann	275 (15.9)	239 (28.2)
Education		
High school or less	273 (15.7)	140 (16.5)
College	1,018 (58.8)	461 (54.4)
Graduate school	432 (25.0)	243 (28.6)
Age at menarche (y)		
< 12	396 (22.9)	230 (27.1)
12–13	1,044 (60.3)	474 (55.9)
≥ 14	291 (16.8)	144 (17.0)
Parity		
Nulliparous	623 (36.0)	251 (29.6)
1	320 (18.5)	130 (15.3)
2	511 (29.5)	293 (34.5)
≥ 3	267 (15.4)	171 (20.2)
Ever use of oral contraceptives		
Never	330 (19.1)	131 (15.5)
Ever	1,401 (80.9)	716 (84.4)
Smoking status		
Never	984 (56.8)	436 (51.4)
Former	470 (27.2)	293 (34.5)
Current	265 (15.3)	117 (13.8)
History of infertility	443 (25.6)	146 (17.2)
Frequency of pelvic examination in past 5 years		
None	62 (3.6)	25 (3.0)
1	133 (7.7)	66 (7.8)
2–3	385 (22.2)	220 (25.9)
≥ 4	1,144 (66.1)	532 (62.7)

Values are number and (percentage). Percentages may not sum to 100 because of missing data.

Table 3. Median Age at Surgery According to Exposure Status and Gynecologic Condition

	Exposed		Unexposed		
	Cases	Median Age (Range)	Cases	Median Age (Range)	*P**
Ovarian cysts					
Functional cyst					
Any size	53	34 (12–48)	24	39 (17–50)	.07
≥ 2 cm	36	34 (12–47)	12	39 (17–48)	.19
Endometrioma					
Any size	22	34 (18–52)	8	32 (17–45)	.52
≥ 2 cm	18	33 (18–49)	4	31 (29–45)	.70
Benign cystic teratoma (dermoid)					
Any size	19	28 (19–50)	6	27 (19–36)	.66
≥ 2 cm	18	29 (19–50)	6	27 (19–36)	.55
Cystadenoma or simple cyst					
Any size	19	29 (19–53)	7	35 (18–45)	.93
≥ 2 cm	14	27 (19–46)	6	36 (18–45)	.68
Paraovarian cysts					
Any size	41	32 (19–53)	9	31 (17–50)	.88
≥ 2 cm	26	32 (19–48)	4	34 (17–50)	.90
Uterine leiomyomata					
Any size	55	41 (20–52)	30	40 (29–50)	.68
≥ 2 cm	37	40 (20–49)	19	39 (30–49)	.85

* Two-tailed *P* from Wilcoxon rank sum test.

interaction terms between DES exposure and age (ie, the underlying time scale), with degrees of freedom equal to the difference in the number of model parameters.

RESULTS

Women who were and were not prenatally exposed to DES were similar with respect to age at menarche, ever use of oral contraceptives, smoking status, and frequency of pelvic examination (Table 2). Exposed women were slightly younger and more likely to report nulliparity and infertility than unexposed women. More than 96% of the cohort was white (data not shown). Median age at surgery was similar among exposed and unexposed women with endometriomas, benign cystic teratomas, paraovarian cysts, and uterine leiomyomata (Table 3). Median age at surgery was lower among exposed women with functional ovarian cysts or cystadenomas than among unexposed women, but these differences were not statistically significant.

Gynecologic tumors that were 2 cm or larger in size were considered the main outcome of interest in the present analysis; smaller tumors are likely to be incidental findings. Age-adjusted incidence rate ratios for exposed women relative to unexposed women ranged from 1.2 for cystadenomas or simple cysts to 3.3 for paraovarian cysts (Table 4). A statistically significant positive association was found only for the latter category (incidence rate ratio = 3.3, 95% CI 1.2–9.5). Incidence rate ratios increased with increasing size for functional ovarian cysts only. Prenatal DES exposure was not associated with risk of uterine leiomyomata (incidence rate ratio = 1.0, 95% CI 0.6–1.7).

Because detection bias may have spuriously elevated the incidence rate ratios, we restricted the sample to women who reported having regular pelvic examinations: 4 or more within the 5-year period before the completion of the 1994 questionnaire (n = 1,676). In this subgroup, associations between DES and specific histologic types were not notably different from those found among all women, with the exception of paraovarian cysts (Table 5). The incidence rate ratio for paraovarian cysts at least 2 cm in size was 9.8 (95% CI 1.3–72.7). Due to the absence of unexposed cases, we could not estimate a meaningful incidence rate ratio for paraovarian cysts at least 5 cm in size. In a separate analysis, we restricted the sample to women with a history of infertility (n = 589), defined as a positive response to the question: "Have you ever seen a physician or other health care provider because you were having difficulty getting pregnant?" These women were likely to have had a complete gynecologic workup, including a pelvic ultrasonography or laparoscopy. Among this subgroup, associations with specific histologic types were considerably weaker, with the exception of the paraovarian cysts: there were 9 cases of any size among exposed women (7 cases at least 2 cm in size) and 0 cases among unexposed women (data not shown).

DISCUSSION

These results are based on a large collaborative cohort study of women with and without documented intrauter-

Table 4. Prenatal Diethylstilbestrol Exposure and Risk of Ovarian Cysts, Paraovarian Cysts, and Uterine Leiomyomata*

	Exposed Cases	Unexposed Cases	Age-Adjusted IRR (95% CI)†
Ovarian cysts			
Functional cyst			
Any size	53	24	1.1 (0.7–1.9)
≥ 2 cm	36	12	1.6 (0.8–3.0)
≥ 5 cm	6	1	3.1 (0.4–26.0)
Endometrioma			
Any size	22	8	1.4 (0.6–3.1)
≥ 2 cm	18	4	2.3 (0.8–6.7)
≥ 5 cm	8	3	1.3 (0.4–5.0)
Benign cystic teratoma (dermoid)			
Any size	19	6	1.6 (0.6–4.0)
≥ 2 cm	18	6	1.5 (0.6–3.8)
≥ 5 cm	12	4	1.5 (0.5–4.6)
Cystadenoma or simple cyst			
Any size	19	7	1.4 (0.6–3.3)
≥ 2 cm	14	6	1.2 (0.5–3.1)
≥ 5 cm	13	3	2.2 (0.6–7.7)
Paraovarian cysts			
Any size	41	9	2.3 (1.1–4.8)
≥ 2 cm	26	4	3.3 (1.2–9.5)
≥ 5 cm	12	2	2.9 (0.7–13.1)
Uterine leiomyomata			
Any size	55	30	0.9 (0.6–1.5)
≥ 2 cm	37	19	1.0 (0.6–1.7)
≥ 5 cm	24	14	0.9 (0.4–1.7)

IRR, incidence rate ratio; CI, confidence interval.

* Analyses of ovarian or paraovarian cysts: exposed = 74,926 person-years, unexposed = 37,752 person-years. Analyses of uterine leiomyomata: exposed = 74,811 person-years, unexposed = 37,418 person-years.

† Adjusted for age in 1-year intervals.

Table 5. Prenatal Diethylstilbestrol Exposure and Risk of Ovarian Cysts, Paraovarian Cysts, and Uterine Leiomyomata Among Women With Regular Pelvic Examination (N = 1,676)*

	Exposed Cases	Unexposed Cases	Age-Adjusted IRR (95% CI)†
Ovarian cysts			
Functional cyst			
Any size	37	16	1.2 (0.6–2.1)
≥ 2 cm	24	8	1.5 (0.7–3.3)
≥ 5 cm	3	1	1.4 (0.2–13.9)
Endometrioma			
Any size	16	4	2.0 (0.7–5.8)
≥ 2 cm	13	3	2.1 (0.6–7.4)
≥ 5 cm	7	2	1.7 (0.3–8.0)
Benign cystic teratoma (dermoid)			
Any size	15	4	1.8 (0.6–5.4)
≥ 2 cm	14	4	1.7 (0.6–5.1)
≥ 5 cm	9	3	1.4 (0.4–5.2)
Cystadenoma or simple cyst			
Any size	16	4	2.0 (0.7–6.0)
≥ 2 cm	11	4	1.3 (0.4–4.2)
≥ 5 cm	10	3	1.6 (0.4–5.9)
Paraovarian cysts			
Any size	31	4	3.8 (1.3–10.8)
≥ 2 cm	20	1	9.8 (1.3–72.7)
≥ 5 cm	10	0	. . .
Uterine leiomyomata			
Any size	38	18	1.0 (0.6–1.8)
≥ 2 cm	27	14	0.9 (0.5–1.8)
≥ 5 cm	18	10	0.9 (0.4–1.9)

IRR, incidence rate ratio; CI, confidence interval.

* Defined as 4+ pelvic examinations in the 5-year period before completion of 1994 questionnaire. Analyses of ovarian or paraovarian cysts: exposed = 49,066 person-years, unexposed = 23,552 person-years. Analyses of uterine leiomyomata: exposed = 49,280 person-years, unexposed = 23,385 person-years.

† Adjusted for age in one-year intervals.

ine exposure to DES. Prenatal DES exposure was positively associated with risk of paraovarian cysts derived from the cephalic portion of the wolffian (mesonephric) or müllerian (paramesonephric) ducts. We found no association between prenatal DES exposure and risk of histologically confirmed uterine leiomyomata or ovarian cysts.

Our findings for paraovarian cysts are consistent with animal studies showing that developing reproductive organs are a potential in utero target for the long-term toxic effects of DES.[8] Exposure to DES during the period of genital tract organogenesis may be expected to change the interaction between müllerian stroma, wolffian stroma, and epithelium.[8] Müllerian stem cells may be programmed during this period and respond to DES exposure with the production of squamous cell or columnar (adenocarcinoma) cell changes.[8] For example, CD-1 outbred mice that were prenatally exposed to DES developed prominent cystic structures of mesonephric origin, as well as abnormalities in paramesonephric-derived tissues.[8,27] Mice not exposed to DES in utero rarely developed these cystic structures.[8,27] A study in humans showed a higher frequency of paraovarian cysts in women exposed prenatally to DES in an infertile population undergoing gynecologic surgery for infertility: 4 of 25 nonexposed women and 8 of 9 DES-exposed women had paraovarian cysts ($P < .02$).[28] Additional studies on DES-treated fetal genital ducts suggest a direct stimulation of the wolffian as well as the müllerian ducts.[29]

The growth of uterine leiomyomata is thought to be influenced by ovarian steroid hormones and locally-derived growth factors.[13,14] Some clinical studies and case reports have found elevations in serum levels of testosterone and prolactin, but not estrogen, among DES daughters (Assies J. Hyperprolactinemia in diethylstilboestrol-exposed women [letter]. Lancet 1991;337:983).[30–31]

Contrary to studies in experimental animals that showed an increased prevalence of smooth muscle tumors in the female genital tract after prenatal DES exposure, the present study does not support an association between prenatal DES exposure and histologically confirmed uterine leiomyomata. A limitation of the study design is that we elicited data only on benign tumors requiring surgery. Although surgery is the standard for confirmation of uterine leiomyomata, ultrasonography is the standard for clinical detection of these tumors. Because histologically confirmed cases represent only 10–30% of cases for whom ultrasound evidence is available, studies limited to surgical cases may spuriously identify risk factors associated with large tumor size, symptoms, or treatment preference.[32] In the animal studies that showed positive associations,[8–10] all mice were screened for the presence of uterine leiomyomata, which would likely identify all tumors regardless of clinical significance. This may be an important methodologic difference between animal and human studies examining this association.

Benign gynecologic tumors are common in the United States,[11] and a large proportion may be asymptomatic.[33] As a result, true cases in the present study could have been misclassified as noncases. If DES-exposed women were more likely to have their tumors detected due to increased medical surveillance, the incidence rate ratio would have been upwardly biased. In our efforts to reduce the influence of detection bias, we restricted our sample to women who reported regular pelvic examination within the 5 years before their completion of the 1994 questionnaire. The results did not change appreciably after this restriction. When the analysis was further restricted to women with a history of infertility, we observed weaker incidence rate ratios observed for all types of cysts except the paraovarian cysts. It is difficult to know whether these results reflect instability of the estimates due to smaller numbers or suggest the influence of detection bias. The pathologist's knowledge of the patient's DES status may have influenced the recording of tumors, particularly those that have minimal or no clinical significance (eg, paraovarian cysts). Although small cysts are commonly overlooked because they are not considered abnormal, such tumors may have been noted if the patient's prenatal medication history was known. To minimize the influence of this bias, only larger lesions were included (2 cm or larger).

In the present cohort study of women with documented prenatal exposure to DES, information on exposure status was ascertained before the diagnosis of gynecologic tumors. The prospective nature of data collection eliminates bias due to differential recall of exposure. However, knowledge of one's DES exposure may have influenced the reporting of gynecologic surgery. Although we validated the self-report of gynecologic tumors through the review of medical records by gynecologists blinded as to the subject's exposure status, the pathologist making the initial diagnosis was rarely blinded in this manner. Thus, greater incidental detection of paraovarian cysts among exposed women could have produced a spurious association with prenatal DES exposure. Another reason for caution regarding the findings on paraovarian cysts is that the lining of the müllerian duct derives from coelomic epithelium, as does the covering of the ovary from which the epithelial cysts arise. It is therefore unlikely that DES would be associated with an increase in cysts of müllerian origin, but not of ovarian epithelial origin.

With the 1994 questionnaire, we achieved follow-up of approximately 80% of the eligible study population. Because losses to follow-up were similar for exposed and unexposed subjects, selection bias is an unlikely explanation of our findings. We were also able to confirm the diagnosis in more than 75% of the cases for whom we obtained medical records. The proportion of confirmed diagnoses did not vary appreciably by exposure status.

In the present study, prenatal DES exposure was associated with an increased detection of paraovarian cysts derived from the müllerian or wolffian ducts, but the clinical significance of these cysts is unknown.[28] Our results do not support the hypothesis that prenatal DES exposure increases risk of uterine leiomyomata or ovarian cysts.

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